May 5, 2005

William S. Stokes, D.V.M., D.A.C.L.A.M.
Director, NICEATM
Executive Director, ICCVAM
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709

Dear Dr. Stokes,

This public comment is delivered in response to Federal Register Notice Volume 70, Number 53, Pages 13513-13514. It addresses the Expert Panel Report on the Evaluation of the Current Validation Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants, March 21, 2005.

First, we would like to compliment the In Vitro Ocular Test method Expert Panel for their time and efforts in reading and evaluating the enormous Background Review Document (BRD) that was generated for the four ocular assays submitted for review. We are also grateful for their discussion at the public meeting in January 2005 and for their thoughtful recommendations in this final report. We recognize that there was a tremendous amount of information and data that had to be processed by the ICCVAM staff (especially Christine Inhof for the BCOP assay) and absorbed by the expert panelists.

With regard to the Final Report itself and the panel's caveats concerning the BCOP that are contained in the Executive Summary, the panelists rightly concluded that histopathology is an important addition to the standard protocol, especially for raw materials and formulations whose chemical behavior in the BCOP assay is not well understood or characterized.

We disagree that the BCOP Assay is not useful in identifying corrosive or severely irritating alcohols and solids. We will address that more completely in our attached comments. We will also address in more detail the issue of whether the BCOP assay "can identify, as well or better than the Draize test, those substances known to cause serious eye injury in humans" (p. ix, Executive Summary).

As to the Expert Panel's suggestions for protocol modifications, we would like to point out that the contract laboratory that we work with has been using an established protocol for a number of years, and our company, among others, has an extensive database at this point. We question whether the panel should be suggesting changes to an established protocol that may invalidate large databases without extensive discussions with experienced user as to the scientific necessity of such modifications.

In addition to comments on the Final Report, we would like to offer comments on the ICCVAM/NICETAM processes as we observed it in the review of these ocular assays. In our opinion, the panel was hampered in several ways from reaching more accurate, definitive conclusions and producing a result that might have been more satisfying to stakeholders that include corporate users, animal protectionist representatives and the regulatory agency representatives.

In the effort to have an objective scientific review, scientists with knowledge and experience about one of the assays were excluded from the panel or a specific subcommittee. This made the evaluation process difficult and less efficient. If NICETAM wants to continue having scientific reviewers with no knowledge of or experience with assays under review evaluate new methods, we respectfully suggest some working discussion sessions with people who conduct these assays on a routine basis before convening the complete panel for final evaluations and recommendations.

There seemed to be a lack of understanding on the part of the panelists concerning the objectives of the meeting. In the future, the panel should be clearly instructed at the outset on the exact purpose of their evaluation (i.e. validation status is met or not).

The time spent on the four methods was not managed well. Far too much time was spent on the first assay reviewed and the end result was a severe time crunch on reviewing the fourth assay, namely the BCOP

assay. In the end, time constraints controlled the process and did a disservice to the review of the assay. The Expert Panels need to have contingency plans in place if time runs out. These decisions are too important to come to conclusions without a full hearing and understanding of the material under review.

Because of the enormous amount of data that had to be assimilated into the BRD, and time constraints once again, the BRD contained a number of factual errors which were addressed in several lengthy public comments to the BRD. Sadly, there was not enough time between the end of the comment period and the meeting of the Expert panel to correct the errors. Thus, the panel did not have correct, or in some cases, complete information concerning the data submitted. Much to our dismay, these same errors or omissions were not corrected in a subsequent presentation at the Society of Toxicology meeting in March 2005.

We respectfully request a careful consideration of our comments in this letter and in the more detailed comments that follow. As we have stated previously, the BCOP assay is a very important part of our product evaluation program. Further, we always welcome any discussion with your and your staff that will serve to clarity technical questions that may arise fro a review of our data.

Sincerely,

Judith E. Swanson
Senior Research Toxicologist

Nicole Cuellar
Senior Research Toxicologist

S.C. Johnson & Son, Inc.
1525 Howe St. – MS 139
Racine, WI 53403

## Comments:

## Executive Summary; BCOP

p. ix. First paragraph. S.C. Johnson regularly uses the BCOP as a part of a weight-of-evidence approach to assess irritation of our nonregistered products (air fresheners, cleaning products and personal care) and label accordingly. We do not believe that the BCOP is only applicable in a tiered testing outlined in the BCOP BRD.

p. ix. First bullet. S.C. Johnson disagrees with the conclusion that the BCOP cannot be used on alcohols and solids without further validation. We submitted data found in Appendix H1 in the BRD (Cuellar et al. (2004) and Appendix H2 (Cuellar et al. (2002) including comparative histology both in the animal and in the BCOP that clearly demonstrates that 3 minutes is more appropriate than 10 minutes for volatile organic solvents. The mode of action of ethanol in both assays involves a quick significant loss of epithelial layer as shown in the histological evaluation in both data sets, which leads to increased tissue injury and/or infection (in-vivo). Organic solvents can react similarly in vivo and in the BCOP by exhibiting overpredictation of irritation. In both data sets, animal recovery was not always consistent with some animals recovering quickly (< 7 days and other(s) not recovering (>21 days).

In regard to solids, S.C. Johnson uses the standard BCOP protocol for solids. We have only one formula over the past 10 years that was less dense than water and thus, floated causing an issue with corneal coverage. Thus, floating test articles have had little impact on our studies. In addition, Sina et al. (1995) was not included in the evaluation of the BCOP. Sina et al. (1995) and Gautheron et al. (1992) was the basis for the development of this assay, which was predicated on solid pharmaceutical intermediates. BCOP BRD should include these data sets in its evaluation.

p. ix. Third bullet. Histological examination is a used routinely by S.C. Johnson for new chemistries or for formulas that are not well characterized in the BCOP. In addition, we use histology for known chemistries with delayed effects or in chemicals where mode of action cannot be easily predicted. It can be used with known chemistries to get a complete picture or to evaluate borderline cases. Generally, histology is not conducted where we have a thorough understanding of our products in the BCOP assay.

p. x. Second bullet. We do not understand why zoonoses, especially BSE is pointed out for this assay. Animal and human tissues are routinely handled in labs globally following appropriate protective safety handling procedures. This assay should not be viewed any differently.

p. x. Third bullet. Concurrent negative and positive controls along with benchmark formulation(s) should be used with each sample that is being evaluated in the BCOP assay. This is standard practice for the use of the BCOP assay at S.C. Johnson.

p. x. Second paragraph. Use on alcohols and solids. See comments above at p. ix. First bullet.

p. x. Third paragraph. Histological perspective. See comments above at p. ix. Third bullet.

p. x. Fifth paragraph. We are in agreement with the Minority opinion on the vote in Section 12.2 (Recommended Standardized Test Method Protocol) of the BCOP by both Expert Panel Members. We feel strongly there was not clear and consistent direction given to the Expert Panel on their objectives in this process. Due to this confusion, time was wasted trying to clarify objectives. Expert Panelists were under enormous pressure to finalize their recommendation on this assay under unrealistic conditions. Initial decisions of the BCOP subgroup were influenced due to the pressures of time and confusion. The ultimate result was a weaker approval of the BCOP as “useful” versus “validated” or “met validation criteria” for identification of severe irritants or corrosives.

p. x. Last paragraph. Validation for use with alcohols and solids. See comments above at p. ix. First bullet.

p. xi. Second paragraph. If validation is needed, it should only focus on the class of chemicals in question.

p. xi. Third paragraph. Any validation should leverage existing animal data.

## III. BOVINE CORNEAL OPACITY AND PERMEABILITY TEST METHOD; Section 1.1 Scientific Basis for the BCOP Method

p. 57. 1.1.1 Mechanistic Basis of the BCOP Method. "…the BCOP test system as outlined in the proposed protocol does not allow one to differentiate the mechanistic cause of the corneal opacity. The BRD mentions only one mechanism of corneal opacity…" Actually the BRD mentions multiple mechanisms of opacity (see lines 214-215, Sec. 1). In addition, histopathology of the cornea is noted as helpful to assess depth and type of injury (Lines 214-215, Sec. 1 and Lines 548-549, Sec. 2). Corneal swelling may be descriptively assessed compared to control by means of histopathological examination and photomicrography.

p. 57. 1.1.2 Advantages and Limitations of Mechanisms/Modes of Action of the BCOP Test Method. Second paragraph. It is imperative that Mauer and Jester's work summarized in Mauer et al. (2002) is included in the evaluation of the BCOP. Mauer et al. (2000) demonstrated that with a series of materials of varying irritation severity that damage to the limbus would be reflected in histological changes in the cornea. This work is a key point of clarification addressing the limbus and scleral vasculature limitation mentioned in the BCOP BRD.

p. 58. 1.1.3 Similarities and Differences of Mechanisms/Modes of Action and Target Tissues Between the BCOP Test Method and Humans and Rabbits. We object to the statement that "the use of the in vivo rabbit test has apparently protected human populations from serious injury for many years." The Draize rabbit eye test only really "protects" if it prevents a product from being sold. It has been documented that the Draize rabbit eye test overpredicts human response but there seems to be no real data quantifying the degree of overpredictiveness. In contrast, Freeberg et al. (1986) did find good correlation between the Low volume Eye Test (LVET, 10 ul of material applied to the center of the cornea) and the human response. The LVET also more closely mimics accidental exposures, than placing 100 ul of test material in the subconjunctival sac of the rabbit eye. Overprediction does not equal scientific credibility, a feature we are striving for in replacing the current whole animal methods.

p. 58. 1.1.4 Mechanistic Similarities and Differences Between the BCOP Test Method, the In Vivo Rabbit Eye Test Method, and/or Human Chemically-Induced Eye Injuries. S.C. Johnson fully agrees with the Expert Panel's recommendation to include Mauer et al. (2002) in the BCOP BRD to support the use of a short term assay for evaluation of long term outcome of a material.

p. 58. 1.1.4. Second paragraph. The importance of the buffering capacity of protective mechanisms is suspect in the use of the current Draize protocol. At the current instillation volume of 100ul, the tearing capacity would be overwhelmed to provide little to no dilution of the material in question. In this respect, the Draize may be similar to the BCOP assay.

## Section 1.2 Regulatory Rationale and Applicability

p. 59. 1.2. S.C. Johnson agrees with the Panel's conclusion to Section 1.2, that "a sufficient mechanistic basis has been established for the BCOP."

p. 59. 1.2.1. Paragraph 1. Similarities and Differences Between Endpoints Measured in the BCOP Test Method and the In Vivo Rabbit Eye Test Method. We note that the BCOP and the Draize tests "measure" corneal effects. It would be more accurate to say the BCOP objectively quantifies corneal effects while the Draize assigns a numerical value based on a subjective scoring system. In addition, iridial and conjunctival effects are likewise graded in the Draize eye test.

The panel notes that the BCOP assay does not give information about iridial and conjuctival effects as well as reversibility of injury and systemic toxicity via the ocular route. We feel that with the addition of histology to the protocol for substances that are not well-characterized, the issue of reversibility disappears. The work of Maurer et al. (2002) shows that the "extent of initial injury is the principal mechanistic factor determining the long-term outcome of ocular injury. Their findings show that in vivo studies are not necessary to assess reversibility of injury.

With regard to ocular systemic toxicity, well-characterized materials such as cosmetics and household products that have been used for years should not be a concern. Evaluating ocular systemic toxicity in vivo should be reserved for newly synthesized molecules. In addition, Mauer et al. (2002) needs to be included in the BCOP BRD to support the point that initial exposure can predict final outcome of injury.

p. 59. 1.2.1 Paragraph 2. We disagree with the sentence stating that "Historical experience indicates the rabbit test has protected human populations using existing scoring systems of the FHSA, EPA and EU. Appendix E shows that there can be considerable variability between regulatory classification systems when classifying a single test material. Likewise, classification within a single regulatory scheme also has the potential to vary widely when a 3 rabbit protocol is utilized. Taking data from a 6 rabbit Draize test and classifying all the possible combinations of 3 rabbits shows wide variation in potential EPA classifications. That is, the possible classifications from single data set may differ by 2 to 3 EPA/GHS categories depending which 3 rabbits are chosen for the analysis from the 6 rabbit set (see Sec. 4.2 of the report). Such variability in classification in one in vivo test within a single regulatory system in a single study implies that the 3 rabbit results would be difficult to replicate from study to study. How then is one to correlate an in vitro method with the in vivo results from 3 rabbit studies, or 6 rabbit studies for that matter?

p.59. 1.2.2 Suggestions Regarding Other Evidence That Might be Used in a Tiered Testing Strategy. First paragraph. We agree that a weight-of-evidence approach should always supplement the employment of the BCOP assay. In evaluating product formulations, we take into account the toxicity of the raw materials used in the formulation, the physicochemical properties of the formulation, information and data on similar formulations, and consumer experience on related products. In addition, we evaluate the potential exposure associated with the product delivery system both in terms of reasonable use and with foreseeable misuse. Exposure scenarios are also considered in determining the in vitro test protocol. That is, does the standard BCOP protocol need to be modified due to some unique formulation characteristic or delivery system?

## 2.0 TEST METHOD PROTOCOL COMPONENTS

p. 61. 2.1.4. Duration of Exposure. Duration of exposure is standardized for specific types of chemicals. For many years, it has been an established practice at S.C. Johnson to use 3 and 10 minute exposure times. We have evaluated 1, 3, & 10 minute exposure times and found that 3 and 10 minutes are the most appropriate for our product types. As mentioned above (p. ix. First bullet), 3 minutes is more appropriate than 10 minutes for volatile organic solvents and standard protocol for solids (4 hrs) is used for solids. In addition, we have discovered that reactive chemicals have a delayed effect, thus requiring modification of the standard solid protocol exposure times. For reactive chemicals, we recommend 10 (human accidental exposure) and 30 (in vivo exposure) minute exposure time with 4 and 20-24 hr post exposure were chosen.

p. 61. 2.1.7. Appropriate Controls and the Basis for Their Selection. S.C. Johnson always utilizes appropriate concurrent benchmark materials along with standard positive and negative controls to evaluate new formulations. A benchmark material is picked based on similar chemistry with previously well-defined toxicity in comparison to the formulation being tested.

p. 62. 2.1.13. Decision Criteria and the Basis for the Algorithm Used. S.C. Johnson does not utilize the classification system as described in the original by Sina et al. (1995) paper to classify severe irritants, i.e., an In Vitro Score of $\geq$55.1. Rather than relying on an absolute cut-off system for the wide variety of products that we evaluate on a daily basis, we believe that comparative toxicity is more reliable basis for classification. This is the reason why S.C. Johnson always utilizes appropriate concurrent benchmark materials, i.e., similar chemistries with previously well-defined toxicity, to evaluate new formulations. Histology is an increasingly important endpoint for us because we believe that depth of injury as seen in histological evaluation is a good predictor of degree and duration of injury as described by Maurer et al. (2002).

## 3.0 SUBSTANCES USED FOR PREVIOUS VALIDATION STUDIES OF THE BCOP METHOD

p. 63. 3.1 Substances/Products used for Prior Validation Studies of the BCOP Test Method. What are examples of the materials in human clinical practice that cause severe eye damage without corneal opacity?

## 4.0 IN VIVO REFERENCE DATA USED FOR AN ASSESSMENT OF TEST METHOD ACCURACY

p. 64. Section 4.2 Interpretation of the Results of the in Vivo Rabbit Eye Tests. Because there can be great variability in classifying a test material both between regulatory systems and within a single regulatory scheme (note remarks on 3 rabbit eye testing the EPA classification system (see p. 59. Sec. 1.2.1-paragraph 2), S.C. Johnson agrees with the concern expressed in Section 4.2 that “these regulatory classification methods may not be adequate for use in evaluating or making distinctions between in vitro methods and their suitability for chemical or product class evaluations.” In addition, such classifications are of little value in predicting human hazard. The histological depth and extent of injury is probably a much better predictor of human hazard.

p. 65. Section 4.6 Accuracy and Reliability of the In Vivo Rabbit Eye Test. Paragraph 1. S.C. Johnson agrees with the statement that “There should be more discussion of the variability of the rabbit data. We agree that “The differences in reproducibility/variability of the in vivo eye data have to be taken into account” (such as, some sort of Uncertainty Factor?) when evaluating BCOP test data. We also agree that the variability that is an inherent characteristic of the Draize eye irritation test must be defined before analyses comparing the results of in vitro data such as BCOP data are correlated to the Draize data. In the end, it seems that the ICCVAM/NICETAM is requiring that new in vitro test methods replicate the variability of the Draize test in order to be validated for future use. We appreciate that the Expert Panel has not taken this position.

## 5.0 BCOP TEST METHOD DATA AND RESULTS

p. 66. Section 5.2 Comparative BCOP Test Method – In Vivo Rabbit Eye Test Data Not Considered in the BRD. We disagree that there is no other data comparing the BCOP assay with in vivo rabbit data. This type of comparative data was submitted but inexplicably not utilized, either in the BRD nor in successive presentations to the expert panel and to attendees of the Society of Toxicology presentation in March 2005 (See BRD; Chapter 3 p. 3-1 and Appendix H –

(H2 and H3). In addition, S.C. Johnson submitted public corrections to BRD regarding the interpretation and use of our data sets used in the evaluation process in Chapter 6 and 7 of the BCOP. It was unclear to us how the data from Swanson and Harbell (2000) was used (See S.C. Johnson BRD comments, p 7, Chapter 6 and p 9, Appendix E-1 &E-2). S.C. Johnson also believes more of the data in Swanson et. al. (1995) should be used in the evaluation of the BCOP. Of the 20 samples, 13 were not used because the instillation volume in vivo was 30ul versus 100ul. Of the 13 samples, 7 resulted in a corrosive/severe classification, thus this 30 ul data should not be eliminated. Sina et al. (1995) should also be used in the evaluation of the BCOP.

p. 66. Section 5.3 Statistical and Non-statistical Approaches Used to Evaluate BCOP Data in the BRD S.C. Johnson agrees that "conclusions relating to test method reliability … drawn from the analyses in BRD Section 7.0 seem sound."

## 6.0 BCOP TEST METHOD ACCURACY

p. 67. Section 6.1 Accuracy Evaluation of the BCOP Test method for Identifying Ocular Corrosives and Severe. Minority opinion. Given our previous remarks on in vivo test data variability, S.C. Johnson is in agreement with Drs. Stephens and Theran that that "consistency" is a much more appropriate term than "accuracy" when comparing in vitro test results to in vivo test results.

## 9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

p. 71. 9.3 Approaches to Expedite the Acquisition of Additional Data. Companies may be hesitant to submit data based on the use and evaluation of the data submitted for the BCOP BRD. As we have stated previously, some of our data was not used at all or incorrectly used in the evaluation of the BCOP BRD. We submitted public comments to address the inaccuracies of the use of our data and the confusion on the interpretation of our data. To date, our comments have not been addressed in the BCOP BRD and/or in subsequent presentations on the BCOP.

## 11.0 PRACTICAL CONSIDERATIONS

p. 72. 11.4 Relative Time Needed to Conduct a Study Using the BCOP Test Method. Unless an animal exhibits signs of distress such as vocalizing, frantic pawing in eye area, agitation, etc., a study is usually continued regardless of the appearance of injury. Since our current regulatory system is based on reversibility of effects, it is in the best interests of the Sponsor to continue a study. Termination at 4 hours would be very unusual (and also would likely result in not marketing the product). We depend on the AALAC-certified laboratory personnel in the contract laboratories to determine the need for termination based on the welfare of the animals on our studies.

Timing is similar from study initiation to receipt of final report between the GLP BCOP and Draize studies.

## 12.0 PROPOSED TEST METHOD RECOMMENDATIONS

p. 72. 12.1.1 Most Appropriate Version of the BCOP Test Method for Use in a Tiered Testing Strategy to Detect Ocular Corrosives and Severe Irritants and/or for Optimization and Validation Studies. S.C. Johnson regularly uses the BCOP as a part of a weight-of-evidence approach to assess irritation of our nonregistered products (air fresheners, cleaning products and personal care) and label accordingly. We do not believe that the BCOP is only applicable in a tiered testing outlined in the BCOP BRD.

p. 73. 12.2 Recommended Standardized BCOP Test Method Protocol. S.C. Johnson regularly uses the BCOP as a part of a weight-of-evidence approach to assess irritation of our nonregistered products (air fresheners, cleaning products and personal care) and label accordingly. We do not believe that the BCOP is only applicable in a tiered testing outlined in the BCOP BRD.

p. 73. 12.2. First bullet. S.C. Johnson disagrees with the conclusion that the BCOP cannot be used on alcohols and solids without further validation. We submitted data found in Appendix H1 in the BRD (Cuellar et al. (2004) and Appendix H2 (Cuellar et al. (2002) including comparative histology both in the animal and in the BCOP that clearly demonstrates that 3 minutes is more appropriate than 10 minutes for volatile organic solvents. The mode of action of ethanol in both assays involves a quick significant loss of epithelial layer as shown in the histological evaluation in both data sets, which leads to increased tissue injury and/or infection (in-vivo). Organic solvents can react similarly in vivo and in the BCOP by exhibiting overpredictation of irritation. In both data sets, animal recovery was not always consistent with some animals recovering quickly (< 7 days and other(s) not recovering (>21 days).

In regard to solids, S.C. Johnson uses the standard BCOP protocol for solids. We have only one formula over the past 10 years that was less dense than water and thus, floated causing an issue with corneal coverage. Thus, floating test articles have had little impact on our studies. In addition, Sina et al. (1995) was not included in the evaluation of the BCOP. Sina et al. (1995) and Gautheron et al. (1992) was the basis for the development of this assay, which was predicated on solid pharmaceutical intermediates. BCOP BRD should include these data sets in its evaluation.

p. 73. 12.2. Third bullet. We do not understand why zoonoses, especially BSE is pointed out for this assay. Animal and human tissues are routinely handled in labs globally following appropriate protective safety handling procedures. This assay should not be viewed any differently.

p. 73. 12.2. Fourth bullet. Histological examination is a used routinely by S.C. Johnson for new chemistries or for formulas that are not well characterized in the BCOP. In addition, we use histology for known chemistries with delayed effects or in chemicals where mode of action cannot be easily predicted. It can be used with known chemistries to get a complete picture or to evaluate borderline cases. Generally, histology is not conducted where we have a thorough understanding of our products in the BCOP assay.

p.74. Minority opinion – Dr. Freeman. S.C. Johnson is in full agreement with the minority opinion. We were disappointed to see the breakdown in the validation process at the Expert Panel meeting resulting under significant pressure to weaken the final conclusions of the Expert Panel sub-committee on the final conclusion of the BCOP. We agree with the suggestions to clarify the objectives and improve the process listed in paragraph on this page. Clear direction should be given to Expert Panel members as to their objectives. Time should not limit directed discussion of validation status of this assay.

In addition, we suggested improvements in development and timing on the BRD. The BRD is a summary document that is used by the Expert Panel to review and to provide final recommendations on the validation criteria of an assay. It is imperative that this BRD document is accurate and comprehensive as possible. Thus, time and resources should be allotted to complete this task. Specifically, each BRD should address public comments especially comments provided by the supplier of the data cited and/or used in the BRD. Any inaccuracies or confusion about data sets should be clarified prior to finalization of the BRD for use by the Expert Panel.

p. 74. Minority opinion – Dr. Theran and Stephans. S.C. Johnson is in full agreement with the minority opinion. We believe that the final report should have concluded that the BCOP was found to be valid and should be moved forward for regulatory review for test method acceptance. Again, the BCOP sub-group concluded that the BCOP had satisfied ICCVAM's validation criteria, thus, valid for severe eye irritants and corrosives. The sub-group was pressured under heated discussion of confusion and time limitations, which resulted in the weakened final conclusion of the "usefulness" of the BCOP assay.

p. 75. 12.3.1. See above in section 12.2 for S.C. Johnson perspective on tiered testing (p. 73. 12.2) idea and use of BCOP on alcohols and solids (p. 73. 12.2. First bullet).

p. 75. 12.3.2. See above in section 12.2 for S.C. Johnson perspective on tiered testing (p. 73. 12.2) idea and use of BCOP on alcohols and solids (p. 73. 12.2. First bullet).

p. 76. Minority opinion #4 & #5. In regard to #4, S.C. Johnson submitted comparative data (BCOP and in vivo data), but it was not utilized, either in the BRD nor in successive presentations to the expert panel and to attendees of the Society of Toxicology presentation in March 2005 (See BRD; Chapter 3 p. 3-1 and Appendix H – (H2 and H3). In addition, S.C. Johnson submitted public corrections to BRD regarding the interpretation and use of our data sets used in the evaluation process in Chapter 6 and 7 of the BCOP. It was unclear to us how the data from Swanson and Harbell (2000) was used (See S.C. Johnson BRD comments, p 7, Chapter 6 and p 9, Appendix E-1 &E-2). S.C. Johnson also believes more of the data in Swanson et. al. (1995) should be used in the evaluation of the BCOP. Of the 20 samples, 13 were not used because the instillation volume in vivo was 30ul versus 100ul. Of the 13 samples, 7 resulted in a corrosive/severe classification, thus this 30 ul data should not be eliminated. Sina et al. (1995) should also be used in the evaluation of the BCOP.

In regards to #5, in vivo variability needs to be taken into account before analyses comparing the results of in vitro data such as BCOP data are correlated to the Draize data.

## REFERENCES:


Cuellar N, Merrill JC, Clear ML, Mun G and Harbell JW (2002). The application of benchmarks for the evaluation of the potential ocular irritancy of aerosol fragrances. The Toxicologist 66(1-S): 243-244.

Cuellar N, Lloyd PH, Swanson JE, Merrill JC, Clear ML, Mun G, Harbell JW, and Bonnette KL. (2003). Evaluating the eye irritancy of solvents in a simple fragrance mixture with the Bovine Corneal Opacity and Permeability (BCOP) Assay. The Toxicologist 72 (S-1): 312.

Cuellar N, Lloyd PH, Swanson JE, Merrill JC, Mun G, Harbell JW, and Bonnette KL. Phase Two: Evaluating the eye irritancy of solvents in a simple fragrance mixture with the Bovine Corneal Opacity and Permeability (BCOP) Assay. The Toxicologist, March 2004: 139.

Freeberg FE, Nixon GA, Reer PJ, Weaver JE, Bruce RD, Griffith JF, Sanders, LW 3rd (1986). Fundamental & Applied Toxicology 7(4): 626-634.

Gautheron P, Dukic M, Alix D, and Sina JF. (1992). Bovine Corneal Opacity and Permeability Test: An In Vitro Assay of Ocular Irritancy. Fundamental & Applied Toxicology 18:442-449.

Gran BP, Swanson JE, Merrill JC, Harbell JW (2003). Evaluating the irritancy potential of sodium percarbonate: A case study using the Bovine Corneal Opacity and Permeability (BCOP) Assay. The Toxicologist 72: 220.

Maurer JK, Parker RD and Jester JV (2002). Extent of initial corneal injury as the mechanistic basis for ocular irritation: Key findings and recommendations for the development of alternative assays. Regulatory Toxicology and Pharmacology 36: 106-117.

Sina JF, Galer DM, Sussman RG, Gautheron PD, Sargent EV, Leong B, Shah PV, Curren RD, and Miller K (1995). A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. Fundamental and Applied Toxicology 26: 20-31.

Swanson JE, Lake LK, Donnelly TA, Harbell JW, Huggins J (1995). Prediction of ocular irritancy of full-strength cleaners and strippers by tissue equivalent and bovine corneal assays. Journal of Toxicology - Cutaneous and Ocular Toxicology 14(3): 179-195.

Swanson JE and Harbell JW (2000). Evaluating the eye irritancy potential of ethanolic test materials with the Bovine Corneal and Opacity (BCOP) Assay. The Toxicologist 54(1): 188-189.